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Inhibition of Generalized Wound Infection in Immunized Rats

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The occurrence of the pyocyanic bacillus in the blood and spleen is compared after intramuscular administration to intact and immunized animals. It is demonstrated that blood clearance is performed by transferring the causative agent to splenic phagocytes. It is suggested that antibodies agglutinize the microorganisms in the primary focus of infection and prevent their entry into the blood.

Key Words: *antibodies; agglutination; bacteremia; sepsis*

Previously it was found that immunization facilitates blood clearance from systemically administered bacteria [5,7]. This was attributed to the opsonizing effect of antibodies on microorganisms and the accelerated uptake of them by liver and spleen macrophages. The potective activity of the antibodies was explained in a similar manner in subsequent papers, for example, upon infection caused by the pyocyanic bacillus (*Pseudomonas aeruginosa*) [6,8]. In real life, as opposed to experiment, the agents causing wound infection usually do not enter the blood directly, but first make contact with tissues and may modulate the probability and duration of bacteremia not only by opsonization but also by agglutination of bacteria. It is more difficult for bacterial aggregates formed as a result of agglutination to cross the tissue-blood barrier [1-3]. In other words, if bacteria have entered the tissue, blood sterility can be maintained by antibodies through opsonization and probably by agglutination. It is not an easy task to differentiate the effects of agglutination and opsonization on bacteremia. We attempted to solve this problem bearing in mind that opsonization facilitates the removal of bacteria from the blood and as-

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TABLE 1. Results of Bacteriological Investigation (Number of Animals; Percent of Number of Animals in Group Given in Parentheses)

Parameter	Period after infection					
	Control				Immunization	
	1 day		3 days		1 day	
Result of bacteriological investigation	*Pseudomonas aeruginosa*	Sterile or other microorganisms	*Pseudomonas aeruginosa*	Sterile or other microorganisms	*Pseudomonas aeruginosa*	Sterile or other microorganisms
Blood	17 (85%)	3 (15%)	10 (36%)	18 (64%)	6 (19%)	25 (81%)
Spleen	18 (90%)	2 (10%)	19 (68%)	9 (32%)	9 (29%)	22 (71%)

Note. Significance of differences (χ^2 – test): blood of immunized animals compared with blood from control animals 1 day after infection ($p<0.01$); spleen of immunized animals compared with control animals 1 day after infection ($p<0.01$). Blood of control animals 3 days after immunization compared with blood of control animals 1 day after immunization ($p<0.01$).

suming that agglutination prevents their entry into the blood.

MATERIALS AND METHODS

Outbred rats weighing 180-200 g (control) were infected (the right calf muscle) with 0.3 ml of a suspension (1-day primary culture) of *Ps. aeruginosa* strain 453 containing 8×10^9 cells/ml of 10% $CaCl_2$ solution [4]. The animals were sacrificed 1 (n=20) and 3 (n=28) days after infection. Bacteriological analysis of the blood and spleen was performed and the titer of agglutinating antibodies to the microorganism was determined.

Thirty-one rats were immunized by 2 intramuscular injections (1-week interval, left calf muscle) of 0.3 ml of *Ps. aeruginosa* (strain 453) suspension (1-day primary culture) containing 2×10^9 cells in normal saline. These rats were infected in the same way 1 week after the immunization and sacrificed 1 day after infection. Blood and spleen were studied as in the control group.

For the determination of the antibody titer 2-fold increasing dilutions of the serum (0.05 ml) were incubated for 1 h at 37°C with 0.45 ml of the bacterial suspension containing 10^9 cells/ml Hanks' solution. After incubation a drop of reaction mixture was viewed in the Goryaev chamber under a phase-contrast microscope at a 200-fold magnification. The ability of the serum to agglutinize the bacteria was characterized by the titer, i.e., the minimal dilution at which bacterial aggregates were formed.

RESULTS

In the control group we did not reveal antibodies to *Ps. aeruginosa* strain 453 on day 1 after infection; on day 3 antibodies were detected in a titer of 1/10-1/640. In the immunized animals the titer was 1/1280-1/5120.

The results of bacteriological study are summarized in Table 1. In the control group the number of animals carrying the microorganism in the blood was significantly lower ($p<0.01$, χ^2 test) on day 3 vs. day 1. The decrease in the number of bacteria in the spleen was much less pronounced. On day 1 after infection, in the immunized group the number of animals carrying *Ps. aeruginosa* in the blood and in the spleen was significantly ($p<0.01$) smaller compared with the control group.

The decrease in the degree of blood contamination with the bacteria on day 3 after infection in the control group coincided with the emergence of antibodies. It is likely that the decrease is caused at least in part by the influence of the antibodies. The organization of the primary focus on day 3 (the development of demarcation and granulation zones) may be another reason for the reduced entry of microorganisms into the blood. Under the chosen experimental conditions the possibility that separation of the primary focus by the demarcation and granulation zones may hamper bacterial contamination of the blood cannot be excluded. However, the fact that in immunized animals the content of microorganisms in the blood and spleen is significantly lower than in the control on day 1 after infection (when the primary focus does not yet have demarcation and granulation zones) proves that the antibodies promote this decrease.

Our results indicate that blood clearance under the influence of antibodies cannot be explained only by opsonization, but agglutination should also be taken into account. This conclusion was made on the basis of the following observations. Opsonization facilitates the removal of bacteria from the blood and their accumulation in the spleen during a certain period which is required for the killing and digestion of the microorganisms. In the control group, a considerable decrease in the blood

bacterial content and a negligible decrease in the spleen bacterial content (compared with day 1 after infection) were observed on day 3. This difference can be attributed to the fact that this was the day on which antibodies appeared. By this time the antibodies can elicit their protective effect on the blood (opsonization, agglutination, or both). However, the spleen content of bacteria changed more slowly. In this organ the effect of antibodies is not pronounced on day 3 after infection. The low bacterial content in the spleen on day 1 after infection testifies to the fact that even in this early period the blood does not now, did not before contain considerable numbers of bacteria. Otherwise, even if the microorganisms had disappeared from the blood they would have accumulated in the spleen. Consequently, the influence of the antibodies manifests itself not in an accelerated clearance of bacteria from the blood, carried out via the spleen, but rather in prevention of the entry of bacteria into the blood, which is achieved by agglutination.

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Morphometric Analysis of Liver Acini in Endotoxicoses Caused by Peritonitis and Gangrene of the Leg

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Morphometric and information analysis is used to quantify the structural changes occurring in the liver and to reveal the specific features of damage to different zones of acini in peritonitis and gangrene of the leg.

Key Words: *liver acinus; peritonitis; gangrene; morphometry; entropy*

Death of patients with endotoxicoses caused by peritonitis, pancreatitis, and gangrene often results from liver failure [2,8,12]. Although there is quite a large body of evidence on the state of the liver, morphological studies are often descriptive and do not take into account regional heterogeneity of the acinus structure [3,7]. There is scant information regarding the specificities of hepatic injury depending on the etiology of endotoxicosis. Morphometry of liver tissue with subsequent information evaluation of the complexity of its structure in a specific pathology may be proposed as a prospective method [4,6].

In this paper we present a comparative morphometric description of liver acini in pronounced endotoxicoses caused by peritonitis and gangrene of

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